


to the original terminology since the undersigned had misinterpreted the disclosure.

The specification has been amended to conform it as originally submitted and claim 14 has been amended to correct an error in order to change polyoxypropylene glycol back to polypropylene glycol.

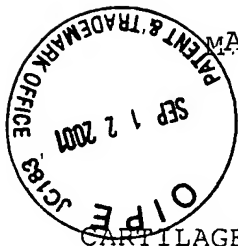
Favorable reconsideration of the application is requested in view of the remarks submitted in the amendment of June 29, 2001 which are not being repeated in order to avoid unduly burdening the record.

Respectfully submitted,
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SPECIFICATION

CARTILAGE/BONE INDUCING MATERIALS FOR PREPARATION

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5 Field of the Invention

The present invention relates to a cartilage and bone morphogenetic repairing material for the treatment of bone fracture and bone defect. In more detail, this invention is concerned with the cartilage and bone morphogenetic repairing
10 material which contains a polyoxyethylene-polyoxypropylene glycol and a bone morphogenetic protein.

Background of the Invention

For repairing cartilages and bones, in addition to auto-
15 plasty, there has been practiced a procedure in which a prosthetic material for defected sites of cartilage and bone composed of a combination of a bone morphogenetic protein and a suitable carrier was imbedded in the defected site. In practicing this, the defected site can be exposed on surgical
20 operation to apply a cartilage and bone repairing material containing a bone morphogenetic protein directly to the defected site, and thus the materials in a solid form such as blocks, sponges, sheets and the like which are easy to handle have been widely applied. Those in a semisolid form such as gels or
25 pastes can also be used. As the carriers which make such solid or semisolid forms applicable, there have been utilized, for example, metals such as stainless or titanium alloys or collagen and hydroxyapatite (HAP) or a mixture thereof.

On the other hand, an attempt has been made to administer a

particular, antigenicity cannot be completely eliminated even when atelocollagen; i.e., collagen from which teropeptide sites are removed, is used (J. American Academy of Dermatology 10, 638-646 and 647-651, 1984 and *ibid.* 21, 1203-1208, 1989).

5 On the other hand, it was reported that biodegradable polymers such as polylactic acid or polylactic acid-glycolic acid copolymers can be used as pharmaceutical carriers (U.S. Patent No. 5,385,887 and Japanese Patent Publication No. 22570/1994). However, the biodegradable polymers are in a solid
10 or semisolid state which may maintain a given form, and in view of this, they are classified as a group of applicable materials to surgical operation. Even if an injectable complex can be prepared using such biodegradable polymers, an organic solvent
15 easily anticipate the problem of inactivation of the active ingredient, a bone morphogenetic protein.

Detailed Description of the Invention

It is an object of this invention to provide a cartilage
20 and bone morphogenetic repairing ^{composition} material, which can overcome the prior art disadvantages or drawbacks as discussed above, which have a high bio-absorption and a good affinity to the active ingredient or a bone morphogenetic protein, and which show the sustained disposition of a bone morphogenetic protein
25 by causing a temperature dependent sol-gel reversible transition with less side-effects such as antigenicity and so on.

The present inventors have made earnest studies on the relationship between the active ingredient, a bone morphogenetic protein, and a carrier therefor in the case of a bone repairing

method without surgical operation and have found that a certain class of polyoxyethylene-polyoxypropylene glycols can show a high bio-absorption, a good affinity to a bone morphogenetic protein and temperature dependent sol-gel reversible transition.

5 The present inventors have prepared a bone morphogenetic material by mixing an aqueous polyoxyethylene-polyoxypropylene glycol solution and a bone morphogenetic protein, which is an injectable liquid at a temperature of from 1°C to 30°C at the time of administration and may be gelatinized at around 37°C within 3 minutes after administration. ^{It has been} They have found that ectopic cartilage and bone morphogenesis are accomplished by administering said material ^{compositely} to mice intramuscularly at the femoral muscle and then retaining a bone morphogenetic protein at the administration sites in vivo, upon which this invention
15 has been completed.

This invention is concerned with a cartilage and bone morphogenetic repairing material which contains a polyoxyethylene-polyoxypropylene glycol and a bone morphogenetic protein.

20 The polyoxyethylene-polyoxypropylene glycol(s) as used herein is a generic name of nonionic surface active agents of a polymer type having less hydrophilic polypropylene glycols as a hydrophobic group and ethylene oxide as a hydrophilic group. It may be feasible to prepare surface active agents having various
25 properties by changing a molecular weight of the polypropylene glycol and a mixing ratio thereof to the ethylene oxide. The synthesizable polyoxyethylene-polyoxypropylene glycols have a molecular weight of the polypropylene glycol in the range of 900-4,000 and a percent by weight of the ethylene oxide in the

total molecule of 5%-90%. For instance, the polyoxyethylene-polyoxypropylene glycol block polymers (ADEKA®) manufactured by Asahi Denka Kogyo K.K. are systematically named according to a molecular weight of polypropylene glycol and a weight ratio of the ethylene oxide to be added and the classification list thereof is shown in Fig. 1.

Industrial utilization of polyoxyethylene-polyoxypropylene glycols includes aperients, ointment bases, artificial blood, coating for tablets, excipients, solubilizers or solubilizing agents for injections and others in the field of pharmaceuticals, in addition to the use as general cleaning agents or antifoamings. In particular, Pluronic F-68 (a molecular weight of polypropylene glycol of 1,750 and an ethylene oxide content of 80%) has a remarkable antihemolytic action and has been marketed in the name of EXOCOPOL® from the Green Cross Corporation as an additive for extracorporeal circulation of blood. It is apparent from the results of toxicity tests using various animals that polyoxyethylene-polyoxypropylene glycols have extremely low toxicity and low irritative property, with no reports on possible side-effects such as antigenicity and so on (Fragrance Journal, 7, 82-87, 1974). The results of toxicity tests are shown in Table 1.

Table 1
Results of acute toxicity tests using ADEKA® Pluronics

<u>ADEKA® Pluronics</u>	<u>Animal species</u>	<u>LD₅₀(g/kg)</u>
L-44, L-62, L-64	Rats	5
F-68	Mice	>15
F-68	Rats, Rabbits, Dogs	No acute toxicity
P-85	Rats	34.6

morphogenetic re~~po~~ring material wherein a concentration of polyoxyethylene-polyoxypropylene glycols as described above in an aqueous solution is about 10-50%. It is known that the reversible phase transition temperature of polyoxyethylene-polyoxypropylene glycols varies in general depending on the concentration of their prepared aqueous solutions, and the polyoxyethylene-polyoxypropylene glycols within the above-mentioned constituent ranges may gelate at around body temperature, i.e., about 37°C at a concentration of about 10-90% in its aqueous solution. As the most preferable example, there is prepared the polyoxyethylene-polyoxypropylene glycol block polymer aqueous solution of 15-30% concentration having a molecular weight of polypropylene glycol of 3,850 and a ethylene oxide content of 70% (Pluronic F-127).

15 The bone morphogenetic protein (BMP) as used herein is the protein having an activity to induce undifferentiated mesenchymal cells to cartilage cells, thereby performing bone morphogenesis.

20 The bone morphogenetic proteins used in this invention include, but are not limited to, a series of proteins belonging to the TGF- β gene superfamily such as BMP-2 to BMP-9 and so on, the protein named MP52, the protein named GDF-5 and the like. Particularly preferable BMP-2 is a protein produced using Chinese hamster ovary (CHO) cells according to the genetic engineering technology reported by Wang, et al. (Proc. Natl. Acad. Sci. USA, 87, 2220-2224, 1990 and U.S. Patent No. 4,877,864), and particularly preferable MP52 is a new protein produced according to the genetic engineering technology suggested by the present inventors (our copending Japanese

of bone fracture or bone defect of human or animal.

Brief Explanation of Drawings

Fig. 1 is a classification figure for ADEKA® Pluronics,
 5 wherein an ethylene oxide content in terms of % by weight in a
 total molecule of a polyoxyethylene-polyoxypropylene glycol is
 indicated on the abscissa, while a molecular weight of the
 component polypropylene glycol in a polyoxyethylene-polyoxypro-
 pylene glycol is indicated on the ordinate.

10 Fig. 2 is soft X-ray photographs of the bone/cartilage
 calcified tissues of the femur in the right hind leg of the
 mouse as obtained by Example 4. The photographs (a) and (b)
 were taken after 2 weeks from the administration of ADEKA®
 Pluronic F-127 solely and ADEKA® Pluronic F-127 containing MP52,
 15 respectively. The apparently blackened parts in the muscle
 indicate ectopically formed bones.

Fig. 3^{and blank} is microscopic photographs of the stained tissues of
 the non-decalcified sections of the femur of the right hind leg
 of the mouse as obtained by Example 4. Formations of bone
 20 matrices and bone matrices together with osteoblasts and of bone
 marrows can be confirmed by von-Kossa staining (a) and Hematox-
 ylin-Eosin staining (b), respectively.

Fig. 4 is a plasmid map of the expression vector of the
 protein MP52 as obtained by Reference Example 1 (2).

Description of the Preferred Embodiments

25 The effects of this invention will be illustratively
 explained by way of the following Examples and Reference
 Examples. However, this invention is not to be restricted by